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Review of Some Toxicology Models to Assess Health Effects of Environmental Pollutants

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REVIEW OF SOME TOXICOLOGY MODELS TO ASSESS HEALTH EFFECTS OF ENVIRONMENTAL POLLUTANTS

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ABSTRACT

The passage of the Toxic Substances Control Act and the recognition over the past several years of the relationship between cancer incidence, other health effects, and environmental agents, prodded EPA-wide interest in test development. Even prior to these occurrences, the EPA Office of Research and Development (ORD) recognized the importance of tests per se to research, regulation, and compliance assurance. ORD thus established efforts along these lines as part of its air and water programs and subsequently in the toxics program, to improve investigative tools to determine and predict health and environmental processes and effects. It is generally agreed that such improvements should strive for increased sensitivity and reliability, and at the same time decrease the cost and amount of time required to obtain results.

The approaches discussed concern the development or refinement of biological testing and testing approaches which can be used by non-ORD groups to predict or assess health effects related to inhalation toxicology (pulmonary and extrapulmonary effects and to a lesser degree new perspectives in teratogenicity testing). Applicability of the research includes those tests or testing approaches which can be used in regulatory or compliance protocols or in guidance for permit issuance.

INTRODUCTION

Much has been said at this symposium concerning using in vitro methodology to assessing the carcinogenic and mutagenic potential of toxic substances in the environment. The potential neurobehavioral and teratogenic effects are also important. I would therefore like to briefly discuss some research approaches considered by the Health Effects Research Laboratory of EPA in North Carolina concerning inhalation toxicology models and teratogenicity testing, which add another facet to the biologic endpoints being considered here. The rationale for our involvement in such research will also be mentioned briefly.

The multidisciplined animal inhalation research program is designed to support EPA's regulatory needs in the areas of criteria and noncriteria pollutants, energy, transportation, pesticides, and toxics. For Air Health and Energy Health, the focus is on the development of sensitive models to measure health effects and/or the use of established models to improve the health effects data base for revisions of existing standards or for the setting of new environmental standards. This research is closely coordinated through various research committees to better meet regulatory milestones.

I. ENVIRONMENTAL POLLUTANTS BEING INVESTIGATED

A. Criteria Pollutants

Inhalation toxicological studies of criteria pollutants, i.e., O_3, SO_x, NO_2, are designed to improve the current data base. Major efforts include, but are not limited to the following:

(1) Strengthening the validity of animal experimental results to assess the health effects in man associated with exposure to gaseous pollutants by use of mathematical models which are predictive of pulmonary dose patterns. Research is being conducted to fill some of the gaps in the modeling data base so that the usefulness of the mathematical model can be expanded to consider absolute differences in doses among species. In this manner, the tremendously large animal toxicological data base will have a more direct impact upon the regulatory decisions of the EPA Administrator.

(2) Since sensitive subpopulations must also be identified and protected, studies are underway to identify these groups and quantify associated health effects. For example, animal models with preexisting lung disease and glucose-6-phosphate dehydrogenase deficiency are being investigated by pulmonary

function and biochemistry as endpoints. Since the young are a significant segment of the population and prior studies indicate they may be at increased risk, they too will be studied for changes in lung morphology, pulmonary function, or host-defense mechanisms.

(3) Since environmental standards are often expressed as time-weighted averages, the complex relationships of concentration, time, and pattern of exposure are being tested with alterations of host-defense systems as the primary endpoints. Studies of the importance of the mode of exposure are of value to determine the need for short-term standards.

(4) To increase the sensitivity of animal models and improve their ability to predict human effects as well as provide improved techniques for human clinical and epidemiological use, new model systems are being developed. These include tests for viral and chronic bacterial disease, extrapulmonary effects, cellular defense systems, pulmonary function in small animals, and lung pharmacology as related to bronchoconstrictor responses.

(5) As future revisions of standards will address pollutant combinations, a significant effort is underway to determine whether pollutant interactions lead to antagonistic, additive, or synergistic effects.

B. Noncriteria Pollutants

To provide data relative to a size-specific particulate standard, inhalation studies are being performed on fine ($\leq$ 2.5 μm) and coarse (2.5-15 μm) mode particles. Fine mode pollutants include $(NH_4)_2SO_4$, NH_4HSO_4, NH_4NO_3, HNO_3, metal sulfates, and metal nitrates. Coarse mode particles were chosen to simulate crustal materials such as kaolin, Fe_2O_3, and $CaCO_3$. Normal and impaired animals will be exposed via inhalation acutely and intermittently to the particles. The following endpoints are being examined: host defense mechanisms, immune system, deposition and clearance, pulmonary function, morphology, lung pharmacology, clinical chemistry, and urinalysis. From these studies, pollutants will be selected for chronic exposures and for pollutant combination exposures.

C. Energy

The goal of this program is to use inhalation toxicology to rank the effects of major forms of energy production (i.e., conventional vs. alternate, oil-fired vs. coal-fired, high sulfur or high vanadium fuel vs. low sulfur or low vanadium fuel). Animals

are exposed to particles collected from plant effluents. Current investigations center on host-defense mechanisms and the immune system.

D. Transportation

Research is also being conducted to support the Office of Research and Development's mobile source research program. The goal of this effort is to assess the carcinogenic potential of materials from diesel engine emissions and to compare their carcinogenicity to that of other environmental substances, such as coke oven emissions, coal tar vapors, and cigarette smoke condensate (all of known epidemiologic significance) as well as to that of similar emissions from gasoline engines. These results will complement those from other concurrent programs and will be of utmost importance in making a scientifically sound assessment of the health effects of the increased use of diesel-powered vehicles.

E. Pesticides and Toxics

For pesticides and toxics, the emphasis is on development, refinement, and validation of model systems suitable for protocol testing. Such an effort is ongoing to continually reduce the time and cost of tests while improving the ability of the models to predict human health risks.

Inhalation toxicological studies of pesticides and toxic substances are a relatively small level-of-effort this fiscal year. Those programs scheduled to be initiated involve validation of host-defense models for toxic substances and pesticides and the pharmacokinetics of inhaled chemicals. It is important to note that many of the tests developed and presently being utilized in the area of air health have now been sufficiently validated and could be applicable for regulatory and/or compliance protocols in the areas of toxic substances and pesticides.

II. METHODOLOGIES

A. Pulmonary Host Defense Mechanisms

1. Description

Many of the methods to be mentioned have been used extensively for testing air pollutants and therefore are defined as validated. It is hypothesized that any chemical that affects the lungs would have the potential for altering pulmonary host defense systems.

The effects of inhaled pollutants on the defense mechanisms in the respiratory tract are one of the major concerns of environmental health science. For the past few years, an acute respiratory disease model has been successfully used and has proven to be exquisitely sensitive to both gaseous and particulate pollutants. This model's sensitivity is due to the fact it reflects the summation of minor alterations in host defenses. In addition to this acute model system, a chronic respiratory disease test has been developed and is available for biological testing.

As is likely to be the case for other major biological endpoints, the major limiting factor for conducting these studies is the ability to conduct inhalation exposures. The biological methods are relatively simple.

2. Acute infectivity model

Test animals are exposed to clean air or pollutants via inhalation for varying lengths of time. After removal from the test atmosphere, all animals are exposed to an aerosol of viable microorganisms (bacteria or virus). Mortality in both the air- and pollutant-exposed groups are recorded over a 15-day holding period in clean air. Comparisons are made of survival time and mortality at the end of the observation period. A recent variation of the model included returning the test animals to the pollutant atmospheres after bacterial exposure. Experiments are underway to modify the model by challenging the animals with bacteria plus virus.

The infectivity model has been used effectively to evaluate the effects of air pollutants on host defense mechanisms against infectious pulmonary disease. From the published literature, it is the most sensitive animal model for O_3 and NO_2. It is also relatively sensitive for particles (metals, sulfates, fly ash, etc.). Its sensitivity is probably due to its ability to measure the net effect of a number or subtle alterations in several host defense mechanisms (mucociliary escalator, alveolar macrophages, immune system, pulmonary edema, etc.).

3. Chronic respiratory infectious model

Normal animals and animals exposed to air pollutants are infected with aerosolized Mycoplasma pneumoniae. Biological indicators involve the measurement of the rate of deposition and clearance of Mycoplasma pneumoniae in the respiratory tract, the histopathological changes during the course of disease development, and changes in the production of protective immunity. A recently developed solid-phase radio immunoassay of high sensitivity will be used to measure the antibody level in the respiratory tract of infected animals under the stress of air pollutants.

These studies are expected to provide essential information concerning the effects of air pollutants on the host defense mechanisms in the respiratory tract during a chronic respiratory infection. The organism used is a human pathogen which is a significant cause of lower respiratory tract disease in children and young adults.

4. Ciliary activity

Several techniques are available, but the one most extensively used for inhaled pollutants is described here. Hamsters are exposed to air or pollutants via inhalation for various lengths of time (a similar model for mice is currently being developed). Ciliary beating frequency of excised tracheas are quantified with a stroboscope attached to the microscope. Morphological correlations are made.

This model has been used to evaluate the effect of pollutants (H_2SO_4, metals, gases) on ciliary beating activity. Alterations are assumed to represent changes in the function of the upper respiratory clearance mechanisms. It should be recognized that clearance is via the mucociliary escalator, and this model measures only one of the two major components. Models to measure actual particle clearance are available for larger animals and are in the process of development and validation for smaller animals. From several studies, it would appear that the ciliary beating frequency model is also suitable as an *in vitro* screening test.

5. Alveolar macrophages

The lungs of animals are lavaged, cells harvested, and examined for the following: number, type, and viability of cells; lysosomal and LDH enzyme activity; phagocytosis; and chemiluminescence.

Since alveolar macrophages are primarily responsible for maintaining sterility in the deep lung, alteration in the cells would have an impact on pulmonary host defense.

6. Pulmonary bactericidal activity

Several major models are available. The simplest is performed in conjunction with the infectivity model and involves quantifying the microorganisms in the lungs at various times after bacterial exposure. A more complex model involves exposing pollutant-exposed mice to aerosols of radiolabeled bacteria and quantifying all bacteria as well as live bacteria present in the lungs at various time periods. Via calculations, the number of bacteria killed can be determined.

Both methods allow measurements of the lungs' ability to control bacterial infection.

B. Pulmonary Function Testing

1. Description

Lung damage resulting from exposure to pulmonary toxicants can be assessed in small animals by using several pulmonary function tests. The lung is a multifunctional organ which can be damaged structurally at different sites. It is important to develop and utilize sensitive tests of pulmonary function which give information on the presence of disease, the site of disease, and, often, the extent of disease in the lung.

There are numerous tests of pulmonary function which have been adapted for use with small laboratory animals, and which indicate the presence of pulmonary disease. These tests indicate the predominant changes which have occurred either in the conducting airways or at the parenchymal level.

2. Tests of airway function

When airways disease is present and the airways are damaged, the affected areas of the lung are usually less well ventilated than in the normal lung. This maldistribution of ventilation can be tested with such tests as the multiple breath nitrogen washout, the single breath oxygen test (single breath nitrogen washout), and estimation of the frequency dependency of compliance. Airways can also become hyperreactive. Hyperreactivity can be tested by changes in dynamic compliance and airway resistance following airway provocation with aerosolized bronchoactive drugs. Measurement of maximal expiratory flow-volume relationships is a good test because changes in the small airways will reduce the maximum rate at which gas can be removed from the lungs, especially at low lung volumes.

3. Tests for disease at the parenchymal level

There are several tests which provide information on the presence of disease in the alveolar region. Estimation of the diffusing capacity of the lung for carbon monoxide indicates the quantity of a test gas which moves from the alveolar air spaces into the blood. This is often reduced in the presence of disease. Lung volumes also change in predictable direction and measurement of the total lung capacity and of the residual volume provide useful information. When surface forces are changed or the lungs become noncompliant ("stiff"), measurement of quasi-static volume pressure relationships will provide helpful information.

These tests have been useful to indicate the presence and severity of disease following acute, subchronic, and chronic exposure of small animals to a variety of inhalable pollutants, both gaseous and particulate.

All of the tests mentioned are similar to tests of pulmonary function which are presently used to evaluate human lung disease. These tests are in routine use with small laboratory animals in several physiology laboratories, and additional tests are under development. Some of the advantages of these tests are: (1) performed in a short period of time on the same individual animal; (2) nondestructive; (3) identify changes in the lungs before such changes are apparent histologically; (4) less expensive and faster than the rigorous morphometric analysis.

Pulmonary function tests may provide very useful data to evaluate the response of animals to toxic substances. They permit early recognition of lung disease, identification of the site of the lesion (e.g., airways, parenchyma, pulmonary capillaries), evaluation of the extent of lung damage and utilize testing procedures now employed in human subjects.

C. Lung Epithelial Damage and Edema

Description

In addition to other functions, the lung acts as a partial barrier between the air and tissues. If this barrier is damaged, materials such as particles and antigens can enter the body more easily and fluid can leak from the tissues into the air spaces (edema). If extensive, edema is a serious health hazard. If minor, it can be indicative of alterations of the epithelial tissues of the lung. The severity of this effect is dependent upon the nature of the alteration.

The rapid method to assess lung epithelial damage is to measure leakage of serum proteins into the lung air spaces. Labeled proteins are injected into the blood and recovered in the lung lavage fluid, or they can be determined as sequestered protein within the lung tissue. Passage of labeled proteins can also be monitored in the opposite direction--from the lung into the blood.

Developmental work is being performed to determine which technique provides the most satisfactory screening test, and to see whether data obtained correlates with long-term pathological indicators of lung damage, such as fibrosis and emphysema. Advantages of the protein leakage model are that it is simple yet quantitative, it is an indicator of damage that at higher levels can be verified morphologically, and it appears to be reasonably sensitive to low level effects.

D. Immune System

1. Description

Since the immune system is involved in a variety of autoimmune diseases and protects man against infectious and neoplastic disease, dysfunction has serious health consequences. Even though the field of immunotoxicity is in its infancy, it is known that a variety of chemicals and drugs can cause effects on this system and that adequate evaluation of toxicity is currently quite difficult and costly. Many good techniques are available, but they need refinement to meet the rigorous quantitative requirements for toxicological research. Toxic substances present an additional complicating factor since either the parent compound or a metabolite may be responsible for the effect. However, current immunological in vitro methods cannot detect the effect of a compound for which the metabolite is the toxic material.

2. Humoral and cell-mediated immunity

Humoral immunity is that portion of the immune system in which the mechanism is via blood proteins (i.e., antibodies are involved). It involves both primary and secondary immunity (properly functioning) for defense against infectious disease. In cell-mediated immunity, as the name implies, cells rather than antibodies are the effector.

The best lung-testing approach would be to use a tier system, so that all chemicals could receive initial, relatively inexpensive testing. Only those compounds that demonstrated potential immunotoxicity would then receive more complete testing. The optimal first tier would involve in vitro exposure. However, for some chemicals, the metabolite is the toxic species, and current in vitro immunological tests would not be effective indicators. However, it is possible that a method could be developed to assay for metabolite effects. Mitogen induced lymphocyte transformation of cells from blood and other organs would be an appropriate model for such development, since it is commonly used as an index of both humoral and cell-mediated immune function and is adaptable to in vitro exposure approaches. Microsomal systems responsible for biotransformation of chemicals would be added to the lymphocyte cultures in a manner similar to that used in the Ames test. Then standard techniques of B and T lymphocyte transformation would be utilized. Chemicals that produced toxic effects after in vitro exposure would then be examined with in vivo exposure methods (oral and/or inhalation). Since circulating lymphocytes can be used in this assay, testing of the chemical could involve in vitro exposure of human cells. Since the relationship of in vitro to in vivo exposure in animals would be known, it would be possible

to estimate human effects. If deemed necessary, epidemiological approaches could be conducted with this same model system.

The following common techniques will also be used: measurement of the number of specific antibody-producing lymphocytes, complete blood counts, enumeration of B and T lymphocytes in blood and lymphoid tissues, circulation of specific antibody titers after primary and secondary antigenic challenge, circulating immunoglobulin titers, assessment of delayed hypersensitivity, and determination of time to endotoxic shock.

The addition of a microsomal activation system to in vitro exposures is in development. All other assays are developed; but since only a few chemicals have been tested in these assays, technique refinement and validation are required. Refinements will primarily be designed to reduce costs of testing.

3. Antineoplastic immunity

A test is being developed that would determine whether a toxic substance compromises host immune defense mechanisms. Mice exposed to the appropriate toxic substances by inhalation or feeding will be inoculated with a low dose of Maloney sarcoma cells (MSC) which are known to cause progressive tumors at high doses but at low doses produces abortive tumors. It appears that at low doses of tumor cells, host immune mechanisms, when unaltered and intact, will cause tumor regression. It is only when the immune response is damaged that a progressive cancer develops. Mice exposed to toxic substances at low doses of Maloney sarcoma cells thus can be used to examine for progressive tumors on the hypothesis that these will develop if the toxic substance injures the immune response.

Other, more developed test systems are available for use. These include cytolytic activity of T lymphocytes and macrophages. For these models, the effect of chemicals on the ability of T lymphocytes and macrophages to kill tumor cells is examined.

These model systems are in development/refinement stages for toxicology testing. Therefore, validation must be completed before the systems are ready for chemical testing. The proposed systems would determine whether toxic substances cause such damage in a rapid (less than 60 days) and inexpensive manner.

E. Cellular Defense Systems

1. Description

A variety of cellular defense mechanisms exist to protect the individual from many insults. Phagocytic cells such as polymorpho-

nuclear leukocytes and macrophages engulf and kill microbes, and engulf and participate in the clearance of other "foreign particles" such as pollutant particles. This inflammatory response also includes chemotaxis (movement of cells towards a stimulus). Inflammation, depending on its site and extent, can be beneficial in the case of destruction of microbes, or can be harmful if the inflammatory cells release too much tissue-destroying enzymes. Therefore, proper bioregulation of inflammation is essential.

2. Chemotaxis

Several model systems exist to measure the movement of cells towards a stimulus. Animals can be exposed and cells recovered from the blood (polymorphonuclear leukocytes and/or macrophages). These cells can also be exposed in vitro. In the foregoing cases, chemotaxis is measured by determining the number of cells which migrate (through a filter or through agarose) towards a chemical stimulus. In another model system, an in vivo measurement of the number of cells that enter the peritoneal cavity is made. In this model, the chemotactic stimulus can be the pollutant under examination or a standard stimulating agent. In the latter case the test would determine whether a pollutant altered in vivo chemotaxis towards the standard stimulus.

Health effects studies with these techniques have been limited. However, after refinement and validation, these test systems could be used for in vitro, acute, subchronic, or chronic exposure tests.

Chemotaxis is the movement of cells, particularly polymorphonuclear leukocytes and macrophages, towards a stimulus. This stimulus can be an infection, dead or dying cells that require clearance, tumors, particles, etc. Thus, chemically induced dysfunction would interrupt a significant defense system. Proper regulation of chemotaxis is essential since the cells which collect at the inflammatory site have a variety of proteolytic enzymes capable of causing tissue destruction.

3. Polymorphonuclear leukocyte function

The functioning of circulating polymorphonuclear leukocytes is assessed by determining their ability to phagocytize and kill bacteria. Radiolabeled bacteria are used to improve quantitation of the system.

These techniques have been used for a few pollutant studies with humans and animals. In vitro exposure systems with animals and/or human cells could be validated as predictors of in vivo effects. If positive in screening tests, in vivo studies could be performed with acute, subchronic, or chronic exposures.

F. Pharmacokinetics

1. Description

Various pharmacokinetic methods have been used successfully to detect pollutant health effects. For example, mixed function oxidase enzymes, responsible for biotransformation of chemicals, are affected by exposure to many pesticies, toxic substances, and other pollutants. Studies are underway to determine whether common air pollutants, such as ozone and nitrogen dioxide, alter the pharmacokinetics of drugs, and thus affect a number of systems involved in processing other chemicals. The pharmacokinetics of the pollutant itself can also be determined to detect possible target sites and provide information to design more intensive studies.

2. Drug-induced sleeping time

Small animals are exposed to pollutants and then drug-induced sleeping time is determined. Drugs such as pentobarbital or hexobarbital are used.

Ambient concentrations of ozone and nitrogen dioxide cause increased sleeping time, thereby illustrating the sensitivity of the model. Other toxicants which perturb mixed function oxidase enzymes are likely to show an effect in this inexpensive method.

This system is a rapid, inexpensive technique which will measure effects of pollutants on the pharmacokinetics of a model substance (i.e., pentobarbital). Since the model reflects the net effect of many complex mechanisms involved in pharmacokinetics, it is a particularly useful screen.

3. Deposition, transport, and clearance

After inhalation of a labeled pollutant, the deposition, transport and clearance are followed. This includes determining the distribution of the metabolites from the parent compound.

Such techniques are routinely used in initial evaluation of a chemical. Generally, exposures are acute, although in some cases where prolonged exposure is likely to alter the deposition, transport, and clearance, the experiment is repeated after subchronic or chronic exposure.

Data from these experiments is useful in models which are designed to extrapolate effective pollutant concentrations between animals and man. Also, knowledge from such studies indicates target sites, which permits improved design of further health effects testing.

G. Postnatal Isozyme Profiles

Description

Isozymes are now widely used as clinical tools in diagnostic medicine as indicators of disease or malfunction. Isozyme profiles have also served a very important role in developmental biology and have permitted the investigation of functional changes which occur during differentiation. However, few developmental studies have employed the neonate or the time interval from birth to adult life even though many developmental changes occur during this period.

In standard teratology testing, the fetus is examined upon dissection. Due to the small size of some organs such as the heart, the examination is cursory. The results are a subjective evaluation of anatomical development with no evaluation of functional development; consequently, the developmental change in the isozyme profiles of two enzymes were explored.

LDH (lactic dehydrogenase) and CPK (creatine phospholinase) were chosen as the two enzymes since their isozyme profiles are used extensively in clinical medicine as indicators of disease or malfunction. Serum and heart tissue were chosen for the initial testing since serum is the media for clinical diagnostic medicine and cardiac problems are a leading health problem of all ages. During neonatal development of the mouse, definite developmental patterns of the isozymes profiles are seen in the serum CPK isozymes and the cardiac LDH isozymes. Changes in these profiles occur after gestational treatment with toxic agents. The changes can be quantified.

The methods for isozyme determinations are routinely used clinically, are readily reproducible, and are of short duration. The reagents and supplies are available in kit form, eliminating preparation time and technician variability.

Postnatal functional evaluation is rarely performed, primarily due to the lack of techniques applicable to the prenatal period. Prenatal toxicology needs extensive development of methods as indicators of toxicity.

H. Model Substrate Assay

Description

Man and animals normally come into contact with pesticides and other environmental toxicants which can potentially alter metabolism in such a way that anomalous toxic metabolites are produced from one or more chemicals present in the mixture. These

metabolites can then either produce lesions directly or, by suppressing the defense mechanisms of the host, promote the toxicity of another chemical.

The hypothesis to be tested is that the generation of unusual alterations in the relative activity of various metabolic pathways of a model substrate can signal toxic interaction. The altered metabolite profiles could potentially be employed as a fingerprint of toxicant exposure. Moreover, xenobiotics, whose metabolites induce the same lesion, may produce metabolite profiles with a common element that may be pinpointed through Discriminant Analysis. Correlation between the occurrence of characteristic metabolite profiles and the type and incidence of specific lesions could further the rational and economical investigation of the induction of these lesions by exposure to multiple toxicants. Thus the "Model Substrate Assay" may provide a useful screen for detecting toxicant combinations to which the general public should not be exposed.

The "Model Substrate Assay" involves repeated exposure of the experimental animals to the toxicants under investigation. After this pretreatment period, the animals receive a single oral dose of a radiolabeled "Model Substrate" whose pharmacokinetic picture has been completely developed. A useful variation in this assay involves the simultaneous administration of the model substrate with toxicants being studied. This permits the determination of changes in the metabolic pathways with time.

Changes in enzyme activity due to disruption of phospholipid membranes, loss of permeability barriers, and loss of local charge effects--which are possible with in vitro assays--are avoided. Enzyme alterations due to accumulation of substrate and/or metabolites are also avoided since normal clearance mechanisms are functional. The assay is economical and practical since it is short term (2-week exposure) and since it predicts potential health hazards that may result from exposure to specific toxicants and toxicant combinations.

I. Fetal Toxicity Indicators in Teratogenicity Bioassays

Description

Studies with standard teratology protocols detect a wide variety of effects within the broad spectrum of developmental toxicity. The formation of gross terata is but one aspect of this spectrum which ranges from fetal death to a large number of variations not considered sufficiently severe to be termed defects. These include supernumerary and wavy ribs, enlarged renal pelvis, enlarged cerebral ventricles, irregular palaine rugae, delayed skeletal ossification, and decreased fetal weight.

The significance of these different anomalous conditions and their role in the risk assessment of chemicals lies in an understanding of the effects of these anomalies on the subsequent health of the postpartum animal. This understanding, as well as the postnatal status of "normal" survivors of affected litters, is generally absent in teratological research.

Our experiments are designed to assess the physiological, biochemical, and morphological status of a variety of organic systems during perinatal development and to use this data as a guide in testing both organ function and reserve capacity in the neonate. By examining the developmental status or organ systems subsequent to exposure to agents which induce the types of developmental toxicity mentioned above, it may be possible to determine whether the effects are reflective of more significant adverse responses than is generally recognized.

We plan to select compounds known to induce various types of developmental toxicity and accurately define the effects of those compounds in a standard teratology protocol. We will use data from this phase of the study to select the optimal dose level that produces clearly demonstrable developmental toxicity and then begin the investigation into postnatal sequelae to such toxic effects. By comparing signs of developmental toxicity with indices of the developmental status of a variety or organ systems, and comparing these, in turn, to the functional capacities of the organ systems in the postnatal animal, we hope to provide the data necessary to better evaluate the outcome of standardized teratology studies.

As a specific example of this approach, the renal system will be examined histologically for the development of glomeruli and renal tubules. In parallel with the histological work-up, the activity of alkaline phosphatase (a marker enzyme for tubular function) will be analyzed. Postnatally, some animals will be challenged with a period of water deprivation and the concentrating ability of the kidneys will be examined.

When all the data from the varous organ systems are complete, we will attempt to correlate the effects observed in standard teratology assays with those observed in our system. In addition, we will attempt to develop an index that describes the significance of the effects to the health and well-being of the affected population.

This study proposes to examine the biological significance of certain forms of developmental toxicity to the animal. When the significance of these effects to the health of experimental animals is understood, we will be better equipped to evaluate data derived from standard teratology protocols in the risk assessment procedure.

J. Postnatal Mouse Teratology Screen

Description

An in vivo teratology screen which is both simple and inexpensive is being developed. This novel approach which utilizes the pregnant mouse relies upon the postnatal growth and viability of pups born to mothers treated during pregnancy as the indicators of toxic effects in vitro. Mothers are treated with a maximum tolerated dose during a critical period of organogenesis (days 8-12 of gestation), and allowed to litter, and their pups' growth and viability recorded for a 72-hour period. To date, some 13 compounds of varying teratogenic potency have been tested and the empirical results indicate an excellent agreement with expected results. All four teratogens tested proved to be positive in the screen. Of the five nonteratogenic compounds tested, four were negative in the screen and only one (Kepone) was positive. Of the two compounds where results in the literature are equivocal in terms of their teratogenic potential, one was doubtful positive ($p<0.10$) and the other was negative. Since this test can be performed for approximately 6 percent of the cost of a standard teratology protocol, requires only 25 percent of the time, and offers the advantage of materno-fetal exchange inherently lacking in all in vitro tests, we strongly feel that the efficacy of this test is being demonstrated.

Teratology shares with all other areas of toxicology a problem which has arisen as a direct result of our current environmental situation. This involves the necessity of testing vast numbers of environmental agents in a short period of time, for a small expenditure of both money and skilled personnel while yielding relevant data. Standard teratology protocols currently in use involve exposing pregnant animals of two or more species to three or more doses of the agent during the period of major organogenesis and subsequently examining the fetuses for both visceral and skeletal anomalies as well as signs of fetal toxicity. While there have been (and will continue to be) modifications in the protocol, it has remained essentially the same as can be seen by examining those proposed by the World Health Organization in 1967, Canadian Ministry of Health in 1973, and the Environmental Protection Agency in 1978. A standard test for a single compound in two rodent species takes at least three months to complete and currently costs $25,000-$35,000. The enormous number of chemicals which are estimated to be in common use, coupled with new legislation, has severely taxed the resources available to use these protocols in testing. The realization that the number of chemicals which should be tested for teratogenic potential was outstripping our ability to do so has resulted in a search for a "screening" system which would identify and prioritize those compounds in need of more detailed testing.

To be of genuine use, a screen should have the following characteristics:

(1) Reliability: The most important factor in any screen is the reliability of the results which are obtained. The screen should mirror, as closely as possible, key processes which act in normal human development. The test system should be sensitive to a variety of agents and should yield neither "false negatives" nor a preponderance of "false positives," since either would negate the utility of the test.

(2) Level of Effort: The screen should be readily obtainable and able to test large numbers of compounds in relatively short periods of time. It should be of simple design, and relatively easy to perform and maintain without constant direct professional supervision.

(3) Economics: The model system must offer economic benefits as compared to standard protocols. Since the current cost of performing a standard teratology assay on a single compound is approximately $25,000-$35,000, it would be desirable for a teratogenicity screen to be considerably less expensive.

The primary difficulties with standard teratology protocols concern the related problems of labor intensity and overall expense. The use of highly trained personnel, along with the laborious fetal examinations (visceral and skeletal), result in the rather expensive test referred to previously. We are proposing a completely noninvasive technique (with the minor exception of a laparotomy on females that fail to deliver) which relies upon any anatomical or function malformations to impair the survival and/or growth of the perinate. It therefore requires little or no labor of a specialized nature and, in essence, substitutes natural mechanisms for the skills of technicians and teratologists. The screen relies instead upon the ability to detect decreases in litter sizes and in neonatal weight at 12 and 60 hours postpartum for its sensitivity. A decrease in the average litter size as measured immediately after birth could be due to embryonic resorptions, perinatal death, and/or cannibalization of malformed pups by the dam (an event known to occur in mice). All of these events can be associated with a fetotoxic action. Less pronounced forms of developmental toxicity may be evidenced by decreased birth weights and by a failure to survive or grow at normal rates during the succeeding 48 hours. The survivorship and growth over this period may be sensitive indicators of congenital cardiac, renal, pulmonary, or other organ function failure. In this sense, the system may be more sensitive than standard teratology protocols in which these functional deficits are missed due to the scarifice before term.

Since the screen does not require laborious and/or specialized techniques, the major costs of this type of study involve animal maintenance. We estimate that a single compound can be evaluated by this method for a cost of $1,600 to $3,000. Furthermore, since the test involves an intact pregnant mammal, the problems raised with the various *in vitro* tests--namely, their potential lack of applicability to a fetal-maternal system--are negated. We have chosen a maximum tolerated dose as the test dose since it would maximize the chance of seeing effects. It is extremely important to note that, contrary to what may be expected, the use of such a dose in no way insures a fetal effect.

As an adjunct to this study, we have also been investigating the possibility that functional deficits may develop at later periods of time in the postnatal animal. This experiment has utilized various parameters which collectively give some indications of the growth, sexual maturity, and behavioral development. The basic protocol is as follows:

On day 6 postpartum, six litters are randomly selected per treatment group, and the numbers of pups are reduced to three males and three females. At 22-23 days of age, one male and one female are tested per litter for their 30-minute reactive-locomotor activity in the residential mazes. At 24 days postpartum, the mothers are removed and the growth and viability of the pups is tracked until 120 days of age. The age at which vaginal opening occurs in females is recorded, as is their age at conception and the litter sizes and weight of the F_1 generation. The relative locomotor activity of the males at 57 days of age, as well as their aggressiveness at 120 days are also determined. At sacrifice, the weights of the kidney, liver, testes, and seminal vesicles are recorded.

The preceding is a summary description of our inhalation toxicology and teratogenicity testing program. Our testing approaches to assess neurotoxicology and genetic toxicology have been described elsewhere in the program.

REFERENCE

Gardner, D. E., E. P. C. Hu, and J. A. Graham, 1979. Proceedings of the Symposium on Experimental Models for Pulmonary Researchers. EPA 600/9-79-022, June 1979.